Germination and Early Growth of Slickspot Peppergrass (*Lepidium papilliferum*) as Affected by Desert Soil Humic Acids

Elisabetta Loffredo,[1] Antonio J. Palazzo,[2] Nicola Senesi,[1] C. Edward Clapp,[3] and Terry L. Bashore[4]

Abstract: Slickspot peppergrass (*Lepidium papilliferum*) is a biennial, or possibly perennial, endemic plant growing in the Southern Idaho high desert in visually distinct small-scale depressions in soils that collect water (so-called *slickspots*). *Lepidium papilliferum* establishes seed banks not germinating the first year but remaining dormant and viable for several years. Humic acids (HA) are universally considered to be the most important, abundant, and biologically and chemically active fractions of soil organic matter and are known to affect plant growth by various mechanisms, depending on their origin, nature, and concentration. The effects of HA in slickspot soils and how they relate to the possibility of being a factor in restoring native plants is only partially known. Thus, the objective of this study was to identify and evaluate the effects of HA isolated from three different layers within the soil profile (silt, vesicular, and clay) from inside a representative slickspot on the germination and early growth of slickspot peppergrass. Furthermore, these effects were tentatively related to the chemical, physicochemical, compositional, structural, and functional characteristics of the HA. Results of statistical analysis showed that both the type and concentration of the three HA examined exert a highly significant or significant effect on the germination and early growth of slickspot peppergrass as a function of the soil depth from which the HA originated in the slickspot. In particular, germination seemed to be enhanced, especially at higher concentrations, by the less hydrophobic HA, rich in oxygen and total sugars, present in the bottom clay soil layer, whereas root growth and shoot growth were positively influenced by the more hydrophobic and probably more polycondensed HA, rich in C, H, N, and phenolic OH present in the top layer rich in silt.

Key words: Slickspot peppergrass, soil humic acids, germination, early growth.

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Slickspot peppergrass (*Lepidium papilliferum*) is a reported "rare" ephemeral endemic plant (Meyer et al., 2005), the distribution of which is restricted to the Snake River plains and Owyhee Plateau regions of Southern Idaho high desert in the United States. In 1999, this plant was listed as a species with "high" threat magnitude and "imminent" immediacy of threat under the Endangered Species Act.

Although the plant is reported as "rare" with "high" and "imminent" threat, there are little, if any, scientific data to substantiate these claims. This species was first proposed for listing as endangered by the U.S. Fish and Wildlife Service in 2002 (Federal Register, 67 FR 46441) and again in 2006 (Federal Register, 71 62078), but both times, *L. papilliferum* was found to be unwarranted for listing.

The name of the plant is derived from its association with slickspots, which are visually distinct small-scale depressions in the soil that collect water (Moseley, 1994; Fisher et al., 1996). The inorganic chemical composition of slickspots has been documented by the USDA-Natural Resources Conservation Service (2003). The organic matter composition has been documented by Palazzo et al. (2008). These sparsely vegetated microsites are created by unusual edaphic conditions and are known by various names such as *natric sites*, *mini playas*, *playettes*, and *slickspots*. Soil accumulation of sodium (Na) may be related to the sparse growth of plants. Because slickspots are essentially areas that accumulate water, the increased Na and electrical conductivity (EC) values may be related to the deeper depth or water-holding capacity of the slickspot. Fisher et al. (1996) noted that the increase in these soil parameters may reduce growth and competition of other plants within the slickspots, but the effects of this increased soil moisture are unknown.

The life cycle of *L. papilliferum* is uncertain. Rollins (1993) reported that *L. papilliferum* is a biennial or possibly perennial species. However, field observations by Meyer (1996), Quinney (1998), and Mancuso (2005) document that this species has both annual and biennial growth forms, but no perennial individuals have been observed. The annual life form of *L. papilliferum* flowers and fruits within the same year, whereas the biennial life form produces basal rosettes the first year and then flowers and fruits the following year (Scholten, 2000; Mancuso, 2005; Meyer et al., 2005). Meyer et al. (2005), who documented the life history of the two life forms of *L. papilliferum*, reported that biennials have higher seed production per plant than annuals.

There is a need for a better understanding of the environmental factors, primarily soil chemistry and moisture, promoting germination and establishment of *L. papilliferum* to allow for better restoration of stands of this species. Increased rate of precipitation is strongly correlated with a higher number of individuals during the growing season (Menke and Kaye, 2006), greater plant dry weights (Meyer et al., 2006), and increase in seed production (Meyer, 1996). Soil chemistry also seems to be a factor in retarding competition of other plants invading the slickspots. Palazzo et al. (2008) found greater concentrations of Na inside as compared with outside the slickspots and greater EC values with depth, which are indications of water storage inside the slickspot. Fisher et al. (1996) noted that the increase in these soil parameters may also limit other species from encroaching the slickspots. These authors also reported that when the Na absorption ratio (SAR) of the soil is greater than 13, the soil condition is classified as *sodic*. Furthermore, they stated that the soil characteristics of the slickspot suggest that edaphic factors have a considerable bearing on where *L. papilliferum* grows and that the lack of competition from

[1]Dipartimento di Biologia e Chimica Agro-forestale e Ambientale, University of Bari, 70126-Bari, Italy. Prof. Elisabetta Loffredo is corresponding author. E-mail: loffredo@agr.uniba.it
[2]ERDC-CRREL, Hanover, NH.
[3]USDA-ARS and University of Minnesota, St Paul, MN.
[4]HQ ACC/A3A, Airspace, Ranges, Airfield Operations Division, Langley AFB, VA.
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other grasses is caused by the high levels of SAR that could provide sites for the *Lepidium* to grow uncontested.

Lepidium papilliferum establishes seed banks that retain viable dormant seeds for years. Meyer (1996) reported that after 4 years, 60% to 70% of original viable seeds in the seed bank were still viable and dormant. At this rate, there will still be living seeds in the ground for at least 7 to 11 years after they fall from the seed pods to the soil (Meyer et al, 2005). Seed germination studies found that *L. papilliferum* seeds stayed alive but dormant for several years, not germinating the first year even under many conditions of light and temperature. Meyer et al. (2005) found that only 10% of the collected seeds germinated in the laboratory, and the seeds exhibited a high degree of cue on nonresponsive primary dormancy.

Humic acids (HA) are universally considered to be the most important, abundant, and biologically and chemically active fractions of soil organic matter (Senesi and Loffredo, 1999). In particular, besides affecting soil fertility indirectly by supplying macronutrients and micronutrients to plant roots, improving soil structure adjacent to roots and governing soil biomass activity (Clapp et al., 2001), HA have been ascertained to affect plant growth by various mechanisms that depend on their origin, nature, and concentration (Chen, 1996; Nardi et al., 1996).

The effects of HA on properties of these soils and whether they are a factor in restoring native plants is only partially known. The HA are known to improve fertilizer efficiency, increase soil cation exchange capacity, and help reduce soil compaction (Palazzo et al., 2008). Thus, the data descriptive of the composition, structure, and functional properties of HA can be used to provide a good basis to help understand the possible effects that HA may have on seed germination and early growth of slickspot peppergrass.

The objective of this study was to identify and evaluate the effects of HA isolated from three different layers within the soil profile (silt, vesicular, and clay) from inside a representative slickspot on the germination and early growth of slickspot peppergrass. Furthermore, these effects were tentatively related to the chemical, physicochemical, compositional, structural, and functional characteristics of the HA.

MATERIALS AND METHODS

Soils and Humic Acids

Soil samples were collected from inside a typical slickspot (15 × 15–cm area) identified on the U.S. Air Force property at the Juniper Butter Range, Owyhee County, ID (U.S. Air Force, 2002). The soils at the sampling site are predominantly Fairylawn fine montmorillonitic, mesic Abruptic Durixesalf (USDA-Natural Resources Conservation Service, 2003). Soils were sampled at the three distinct typical layers within the slickspot: silt (0, about 2.5 cm), vesicular (about 2.5–3.5 cm), and clay (about 3.5–15 cm). Soils from each layer were air-dried and ground to pass through a 2-mm mesh screen before analysis and HA isolation. Details on soil analytical properties can be found in a previous article (Palazzo et al., 2008) and are typical of a slickspot.

The three HA samples studied in this work were isolated from the silt (HAsi), vesicular (HAves), and clay (HAcl) layers of soil according to a modified International Humic Substances Society method using 0.5 *M* NaOH (Swift, 1996). The extract was membrane filtered (0.2-μm pore size), the filtrate was adjusted to pH 1 (6 *M* HCl), and the precipitated HA were centrifuged, dialyzed, and freeze-dried. The HA isolated were characterized by elemental analysis, and Fourier transform infrared (FT IR), emission/excitation matrix fluorescence, and nuclear magnetic resonance spectroscopies by Palazzo et al. (2008).

Germination and Early Growth Experiments

Peppergrass seeds used in this study were collected from live plants in the field at the Juniper Butter Range. Because of the high rate of seed dormancy of this species, the evaluation of a number of preliminary alternative seed treatments was necessary to determine the best means to break dormancy and obtain consistent germination rates. The various seed pretreatments selected for testing were the following: (i) soaking (SK) in distilled water for 24 h; (ii) scraping (SC) dry seeds with fine sand paper; (iii) sterilizing (ST) with 0.2% sodium hypochlorite solution for 15 min; (iv) piercing (PC) with steel needle; (v) shaking with 4 mL of suspended activated charcoal (AC) at 10 mg L^{-1} for 1 h; (vi) soaking in pure acetone (A) or 0.2% (vol/vol) acetone/water for 15 min, then washing with distilled water; and (vii) various combinations of the previously mentioned treatments (Table 1). After each pretreatment, 30 peppergrass seeds were placed in Petri dishes on filter paper, and bidistilled water was added. All wetted seeds seemed to be coated by a thick gelatinous layer. The Petri dishes containing the seeds were kept under diffuse light of tungsten lamps in a thermostated chamber at a constant temperature of 25°C for 5 days. Only the treatment (SK + PC wet) was replicated seven times for a total of 210 seeds, whereas all the other treatments were not replicated. The number of germinated seeds was counted after 7, 13, and 23 days (Table 1).

TABLE 1. Number of Germinated Seeds (30 Seeds per Petri Dish) After Different Periods

	No. Germinated Seeds			
Treatment	**7 Days**	**13 Days**	**23 Days**	**Total No.**
SK	0	0	2	2
SC dry	1	8	0	9
SC dry + SK	5	5	0	10
SC dry + ST	0	2	0	2
SC dry + ST + SK	0	0	0	0
SC wet	0	0	0	0
SC wet + SK	4	0	0	4
SC wet + ST	1	0	0	1
SC wet + ST + SK	3	0	0	3
PC wet	6	0	0	6
SK + AC	1	0	0	1
SC + SK + AC	3	0	0	3
SK + 0.2% A	0	0	0	0
SC + SK + 0.2% A	2	0	0	2
SK + pure A	0	0	0	0
SC + SK + pure A	0	0	0	0
SK + PC wet	5†	6†	0	11†

Soaking (SK) in distilled water for 24 h.

Scraping (SC) with fine sand paper.

Sterilizing (ST) with 0.2% sodium hypochlorite solution for 15 min.

Piercing (PC) with steel needle.

Shaking for 1 h in 4 mL of 10 mg L^{-1} activated charcoal (AC) suspension.

Soaking for 15 min in pure acetone (A) or 0.2% (vol vol^{-1}) acetone/water and washing with distilled water.

†Total number counted in 7 Petri dishes (210 seeds).

TABLE 2. Some Chemical and Physicochemical Parameters (on a Moisture- and Ash-Free Basis) of HA Examined (Palazzo et al. 2008)

Parameter[a]	C	H	N	O	Total Sugars	Total Amino Acids		Total Acidity	COOH	Phenolic OH
Sample	g kg^{-1}				μg mg^{-1}	mmol mg^{-1}	RFI	meq g^{-1} C		
HAsi	575	62	60	301	26.4	1.705	173	5.26	3.35	1.91
HAves	564	55	52	327	26.7	1.134	265	5.26	3.58	1.68
HAcl	559	50	51	337	27.3	0.994	354	4.67	3.33	1.34

On the basis of the results of the pretreatments previously described, the combined pretreatment that yielded the best germination results consisted of scraping (SC) the dry seeds with fine sand paper and then soaking (SK) them in distilled water for 24 h before starting germination experiments. Thus, this pretreatment was selected and used in the main experiment.

Sets of 30 seeds pretreated as previously described were placed in Petri dishes on filter paper. Suspensions of each HA at concentrations of 10, 50, and 200 mg L^{-1} or bidistilled water (control) were added. Each HA was first dissolved in a few drops of KOH and then brought to the final volume with bidistilled water, reaching a final pH ranging from 6.5 to 6.8. The Petri dishes containing the seeds were kept for 5 days in a thermostated chamber under diffuse light of tungsten lamps at a constant temperature of 25°C. No seeds germinated under these conditions, thus the gelatinous outer layer of each seed was pierced (PC) with a steel needle, and the pierced seeds were allowed to germinate for 6 more days. Then, the young seedlings were removed and counted, and the lengths of the primary root and shoot were measured. All experiments were conducted in four replicates.

For the early growth experiment, peppergrass germinated seedlings were transplanted into plastic pots containing cottonwool soaked with 8 mL of the Nitch nutrient solution in the absence (control) or presence of each HA at concentrations of 10, 50, and 200 mg L^{-1}. The pH of all solutions was previously adjusted to a value of 7.3 with a solution of KOH. The pots were covered with Parafilm and placed in a Fitotron growth chamber where seedlings were allowed to grow for a period of 24 days under the following conditions: (i) photoperiod of 12 h; (ii) temperature of 25°C during the illumination period and 20°C during the dark period; and (iii) constant humidity of 74%. At the end of the experiment, the length of roots and shoots were measured. All experiments were conducted in four replicates. It was not possible to measure the fresh and dry weights of shoots and roots because the amounts obtained were very small.

All germination and early growth data were analyzed statistically by one-way analysis of variance (ANOVA) and the least significant differences (LSD) test. The mean values obtained for the germination percentage and the lengths of primary root and shoot and 24-day grown roots and shoots were statistically correlated with a number of chemical and functional properties of the HA, including the contents of the major elements, acidic functional groups, neutral sugars, and total amino acids, and relative fluorescence intensity.

TABLE 3. Significance Level (F Value) Resulting From One-Way ANOVA of All Data Obtained for Each Parameter Measured for Slickspot Peppergrass

Parameter	F
Germination	2.48[†]
Primary shoot length	4.30[‡]
Primary root length	2.23[†]
Shoots length	3.73[‡]
Roots length	3.26[‡]

[†]$P = 0.05$.
[‡]$P = 0.01$.

RESULTS

Humic Acids

In a previous work, Palazzo et al. (2008) characterized the HA isolated by chemical methods and physicochemical techniques, showing that the soil layer influenced the elemental and functional composition of the HA examined. In particular, with increasing depth of soil, the C, H, N, phenolic group, and total amino acid contents generally decreased slightly, and the O, total acidity, and total sugars contents increased slightly (Table 2).

Furthermore, some semiquantitative differences between HA from different layers in the relative intensity of some FT IR absorptions were apparent (Palazzo et al., 2008). In particular, the aliphatic group absorptions decreased in the order: HAsi > HAves > HAcl; the relative intensity of peaks preferentially ascribed to various carbonyl, especially carboxyl, groups increased slightly in the order: HAsi < HAves < HAcl; and the peak preferentially ascribed to amides was present only in the spectrum of HAsi. In general, FT IR results suggested a decreasing aliphaticity and increasing presence of COOH groups in HA downward for the soil layers.

The relative fluorescence intensity (RFI) calculated from the emission/excitation matrix fluorescence spectra (Palazzo et al., 2008) differed between the HA investigated and generally

TABLE 4. Effect of HA at Different Concentrations on Seed Germination (Percentage of Germinated Seeds ± SE for Four Replicates) of Slickspot Peppergrass Measured Immediately Before Transplanting

Treatment	Concentration, mg L^{-1}	Germination, %
Control (H_2O)		8.3 ± 1.9
HAsi	10	11.7 ± 1.9
	50	8.3 ± 1.9
	200	10.8 ± 0.7
HAves	10	13.3 ± 2.0
	50	13.3 ± 2.0
	200	10.8 ± 1.4
HAcl	10	8.3 ± 1.9
	50	16.7 ± 1.7[†]
	200	18.3 ± 3.0[†]

S.D. ($n = 4$) are also indicated.
[†]$P \leq 0.05$, according to the LSD test.

TABLE 5. Effect of HA at Different Concentrations on the Length (cm ± SE for Four Replicates) of Primary Shoot and Primary Root of Germinated Seeds of Slickspot Peppergrass

Treatment	Concentration, mg L^{-1}	Shoot Length	Root Length
Control (H_2O)		0.63 ± 0.07	0.96 ± 0.05
HAsi	10	0.65 ± 0.06	1.29 ± 0.22
	50	1.01 ± 0.02*	1.44 ± 0.03**
	200	0.60 ± 0.08	1.70 ± 0.50**
HAves	10	0.48 ± 0.07	1.06 ± 0.26
	50	0.47 ± 0.11	1.06 ± 0.05
	200	0.48 ± 0.10	1.03 ± 0.20
HAcl	10	0.40 ± 0.09	0.80 ± 0.02
	50	0.40 ± 0.05	0.87 ± 0.15
	200	0.59 ± 0.05	0.98 ± 0.05

S.D. ($n = 4$) are also indicated.

**$P \leq 0.01$; *$P \leq 0.05$, according to the LSD test.

tended to increase slightly in the order: HAsi < HAves < HAcl (Table 2). In general, the ^{13}C-CP/MAS spectra (Palazzo et al., 2008) showed that HAsi contained larger amounts of nonpolar alkyls and peptide structures than HAves and HAcl. The sample HAcl contained significant aromatic structures and *O*-aromatic groups. Major differences appeared between the spectra of HAsi and HAcl, whereas the spectrum of HAves had many similarities with that of HAsi.

Germination

A small number of slickspot peppergrass seeds germinated after 7, 13, and 23 days (Table 1). The low rate of germination was primarily caused by the high rate of seed dormancy with this plant. The variation in germination rate showed that some of the pretreatments were effective in promoting seed germination. The higher numbers of germinated seeds were obtained after 13 days in the following conditions: (i) SC dry + SK (10 seeds germinated) and (ii) SC dry (nine seeds germinated). The SK experiment produced a late (23 days) and very small (two seeds) germination rate, whereas most of the other treatments yielded limited germination in the first 7 days, which then stops. Thus, in the main experiment, the seeds were germinated using the combined conditions previously described in the Materials and Methods section.

One-way ANOVA of all data obtained for seed germination in the main experiment indicated that the different treatments caused highly significant ($P \leq 0.01$) differences for the lengths of primary shoot and 24-day shoots and roots and significant differences ($P \leq 0.05$) for germination percentage and primary root length (Table 3).

Numerical data of the effects of the three HA examined at three different concentrations on the germination parameters of slickspot peppergrass are shown in Table 4 (absolute germination percentage) and Table 5 (absolute length, in centimeters, of primary shoot and root of germinated seeds) and in Figs. 1 and 2 (same parameters expressed as percentages of those of the control treatment, assumed to be 100%). With respect to the control, any HA treatment either had no influence on or stimulated germination and primary shoot and root lengths of peppergrass. Data obtained indicated that only HAcl at concentrations of 50 and 200 mg L^{-1} promoted the germination percentage, whereas no significant effect on germination was evident for any other treatment. A statistically significant stimulation of primary shoot length and root length is exerted only by HAsi at the concentration of 50 mg L^{-1} ($P \leq 0.01$) for shoot and at 50 and 200 mg L^{-1} for root ($P \leq 0.05$).

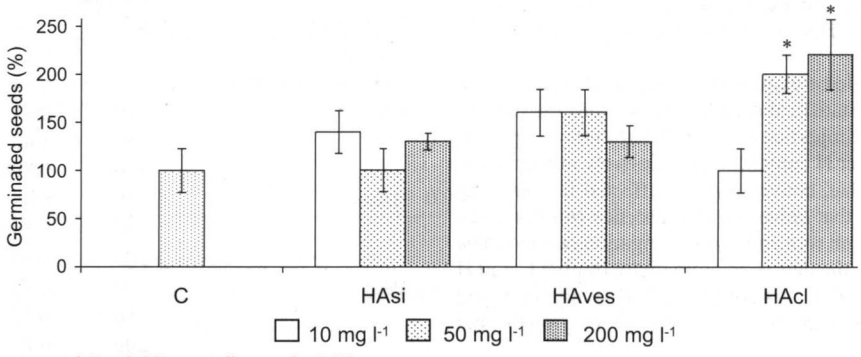
Early Growth

One-way ANOVA of all experimental data obtained for shoot and root length after 24 days of growth showed the existence, with respect to the control (nutrient solution alone), of a highly significant difference ($P \leq 0.01$) as a function of either the HA type or HA concentration (Table 3).

Numerical data obtained for the effects of the three HA examined at three different concentrations on shoot and root lengths of slickspot peppergrass after 24 days of growth are shown in Table 6 (absolute lengths, in centimeters) and in Fig. 3 (lengths expressed as percentages of those of the control treatment, assumed to be 100%). Data obtained show that HAsi stimulated shoot elongation highly significantly at 50 mg L^{-1} and root elongation significantly or highly significantly at 50 and 200 mg L^{-1}, respectively. A significant increase of roots length was also caused by HAves at 10 mg L^{-1} and HAcl at 200 mg L^{-1}.

Structure-Activity Relationships

Tables 7 and 8 show the correlation coefficients calculated between the variation (%) caused by the various HA treatments,



* $P \leq 0.05$, according to the LSD test.

FIG. 1. Effect of HA at different concentrations on the number of germinated seeds expressed as percentages of control treatment (100%). The vertical line on each bar indicates the SE for four replicates.

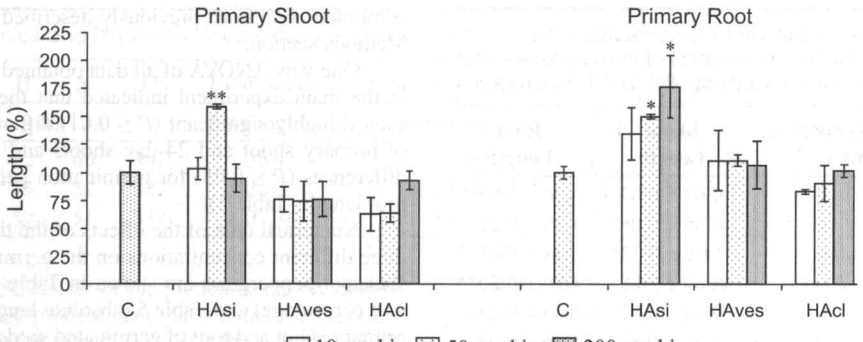


FIG. 2. Effect of HA at different concentrations on primary shoot and root length of germinated seeds, expressed as percentages of control treatment (100%). The vertical line on each bar indicates the SE for four replicates.

with respect to the control, of germination percentage and lengths of primary root and shoot and roots and shoots of plants grown for 24 days and the main chemical and functional properties of HA (Table 2). Data obtained indicated that all correlations that were positive for germination were negative for the elongation of roots and shoots, and *vice versa* (Tables 7 and 8). The germination percentage seemed to be negatively correlated with the contents of C and H ($P \leq 0.05$ and $P \leq 0.01$, respectively, at the mean concentration and 50 mg L^{-1}), N, total amino acids, total acidity ($P \leq 0.001$ at 200 mg L^{-1}), and phenolic OH, whereas it is positively correlated with the contents of O and total sugars and to the RFI. In all cases, opposite relationships occur for the lengths of primary and 24-day-grown roots and shoots (Tables 7 and 8).

DISCUSSION

Results of statistical analysis showed that both the type and concentration of the three HA examined exerted a differentiated highly significant or significant effect on the germination and early growth of slickspot peppergrass. In particular, among the HA examined, only HAcl at 50 and 200 mg L^{-1} caused a statistically significant increase of the germination percentage, whereas no influence was exerted by the other two HA at the three concentrations examined. However, HAsi significantly stimulated the growth of primary shoot at 50 mg L^{-1} and that of primary root at 50 and 200 mg L^{-1}, whereas HAcl did not produce any statistically significant modification. These results thus suggested that germination and early growth responded differently to the same HA or were promoted by HA with different properties.

These effects could be explained by their relationships with some HA properties. Statistical correlations between the percent germination and HA properties suggested that the hydrophobic character of HA (higher H content) may negatively influence the germination process that, conversely, was affected favorably by their presence in HA that enhanced fluorescence (higher RFI). The stimulating effect on root and shoot elongation seemed to be positively related with the degree of aromaticity (higher C and H content, less O and total sugars content and RFI), higher N and amino acid content, especially for primary shoot growth, and phenolic OH content.

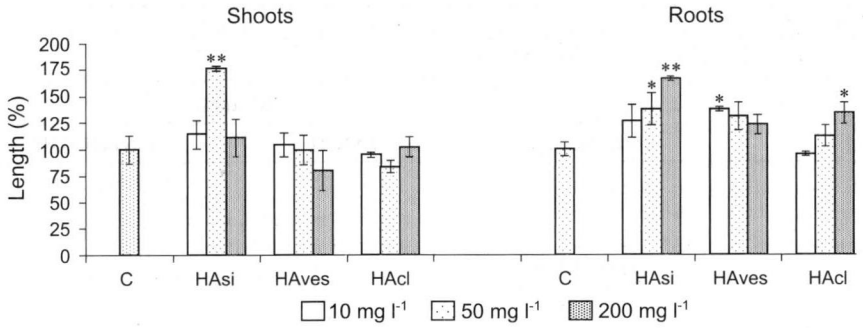
Stimulation of shoot and root elongation after 24 days of growth was exerted mainly by HAsi, but also by HAves and HAcl at low and high concentrations, respectively. Several studies have reported that HA were able to enhance germination and growth of plants, and that this effect was greatly dependent on the origin, nature, and concentration of HA, and conditions used in germination and growth experiments (Chen and Aviad, 1990; Chen, 1996; Nardi et al., 1996). The data reported in this article clearly indicated that HA properties related to their parent soil layer were important in HA biological action. Data of structure-activity correlations obtained for the growth period of 24 days were similar to those measured for the germination period, which suggested that the relevant parameters remain generally the same during the whole plant growth.

The data reported here illustrate the different effects of HA on peppergrass growth as a function of the soil depth from which the HA originates in the slickspot. However, the possible causes of emergence of peppergrass from slickspots are still obscure. Because slickspots are essentially areas that accumulate water, the increased EC and water-holding capacity (*P*) values may be related to the deeper depth of the slickspot. Whether the greater number of plants in soils with a higher EC and P is related to lack of competition from other plants, or higher availability of HA to plants, or increased soil moisture is unknown.

TABLE 6. Effect of HA at Different Concentrations on the Length (cm ± SE for Four Replicates) of Shoots and Roots of Slickspot Peppergrass Measured After 24-Day Growth

Treatment	Concentration, mg L^{-1}	Shoot Length	Root Length
Control (nutrient solution)		0.81 ± 0.10	1.27 ± 0.08
HAsi	10	0.92 ± 0.11	1.60 ± 0.20
	50	1.42 ± 0.29*	1.75 ± 0.19**
	200	0.89 ± 0.14	2.11 ± 0.41*
HAves	10	0.84 ± 0.09	1.74 ± 0.32**
	50	0.80 ± 0.11	1.65 ± 0.17
	200	0.64 ± 0.17	1.56 ± 0.13
HAcl	10	0.76 ± 0.03	1.21 ± 0.32
	50	0.67 ± 0.05	1.42 ± 0.13
	200	0.82 ± 0.07	1.70 ± 0.13**

S.D. ($n = 4$) are also indicated.
**$P \leq 0.01$; *$P \leq 0.05$, according to the LSD test.



** $P \leq 0.01$; * $P \leq 0.05$, according to the LSD test.

FIG. 3. Effect of HA at different concentrations on the length of shoots and roots expressed as percentages of control treatment (100%) measured after 24-day growth. The vertical line on each bar indicates the SE for four replicates.

Maintaining a persistent soil seed bank is crucial to population viability, especially during periods of low precipitation. Our data show that the scratched or abraded seeds respond to the HA treatments. Whether HA can promote seed density, through the production of seeds of parent plant material, would be a benefit in maintaining the species. Meyer et al. (2005)

TABLE 7. Correlation Coefficients Calculated Between the Variation Percentages With Respect to the Control Produced by the Three HA for the Various Parameters and HA Properties

	Parameter†					
HA Concentration, mg L^{-1}	**C**	**H**	**N**	**O**	**Total Sugars**	**Total Amino Acids**
Germination percentage						
10						
50	−§	−0.9999**	−	+‖	+	−
200		−				
Mean‡	−	−0.998*		+	+	−
Primary root length						
10	+	+		−	−	
50	+	0.9999**	+	−	−	+
200	+		+	−		+
Mean‡	0.9999**	+	+	−0.9999**		+
Primary shoot length						
10	0.9999**	+	+	−0.9999**		+
50	+	+	0.9999*	−		0.997*
200						
Mean‡	+		0.999*	−		+
Root length after 24-day growth						
10						
50		+			−0.998 *	
200						
Mean‡	+	+			−	
Shoot length after 24-day growth						
10	+	+			−	
50	+	+	0.997*	−0.997*		1.0000***
200						
Mean‡	+		0.999*	−		+

†Data in Table 1.

‡Mean of variations (%) obtained at 10, 50, and 200 mg L^{-1}.

§Negative correlation coefficient, absolute value between 0.95 and 0.99.

‖Positive correlation coefficient between 0.95 and 0.99.

*Statistically significant at $P \leq 0.05$ ($n = 3$; df, 1).

**Statistically significant at $P \leq 0.01$ ($n = 3$; df, 1).

***Statistically significant at $P \leq 0.001$ ($n = 3$; df, 1).

TABLE 8. Correlation Coefficients Calculated Between the Variation Percentages With Respect to the Control Produced by the Three HA for the Various Parameters and HA Properties

	Parameter[†]			
HA Concentration, mg L^{-1}	**Total Acidity**	**COOH**	**Phenolic OH**	**RFI**
Germination percentage				
10				
50			−[§]	+[‖]
200	−1.0000***			
Mean[‡]			−	0.9999**
Primary root length				
10			0.997*	−0.999*
50			+	−
200				
Mean[‡]				−
Primary shoot length				
10				−
50				
200		**−0.997***		
Mean[‡]				
Roots length after 24-day growth				
10	+			
50	+		+	−
200				
Mean[‡]			0.999*	−0.997*
Shoots length after 24-day growth				
10			0.9999**	−
50	+			
200				
Mean[‡]				

[†]Data in Table 1.
[‡]Mean of variations (%) obtained at 10, 50, and 200 mg L^{-1}.
[§]Negative correlation coefficient, absolute value between 0.95 and 0.99.
[‖]Positive correlation coefficient between 0.95 and 0.99.
*Statistically significant at $P \leq 0.05$ (n = 3; *df*, 1).
**Statistically significant at $P \leq 0.01$ (n = 3; *df*, 1).
***Statistically significant at $P \leq 0.001$ (n = 3; *df*, 1).

sampled 189 cm^2 in each of three locations ranging from heavily disturbed to high-quality sites and found that the seed bank size ranged from 0 to 18 viable seeds per cm^2.

CONCLUSIONS

Results obtained in this work highlighted the important ecological role of soil organic matter and the need of preserving soil organic fertility for plant maintenance. Humic substances, which represent the most stable organic reservoir of soils, are known to exert not only a general positive biological action, but also to possess a specific and differentiated influence on the various plant growth processes. In particular, the three HA tested in this study differently affected the germination percentage and root and shoot growth of slickspot peppergrass. The first process seemed enhanced by the less hydrophobic HA present in the bottom soil layer rich in clay (HAcl), especially at higher concentrations, whereas root and shoot growths were positively influenced by the more hydrophobic and probably more polycondensed HA present in the top layer rich of silt (HAsi).

More research efforts are needed to possibly elucidate the biochemical mechanisms underlying the effects of soil HA, also considering other soil components, on the germination and early growth of slickspot peppergrass in these soils.

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